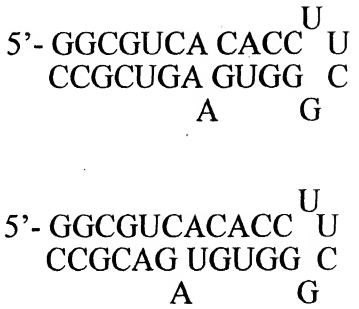


Figure 1. Sequence and structure of 27mer RNA target

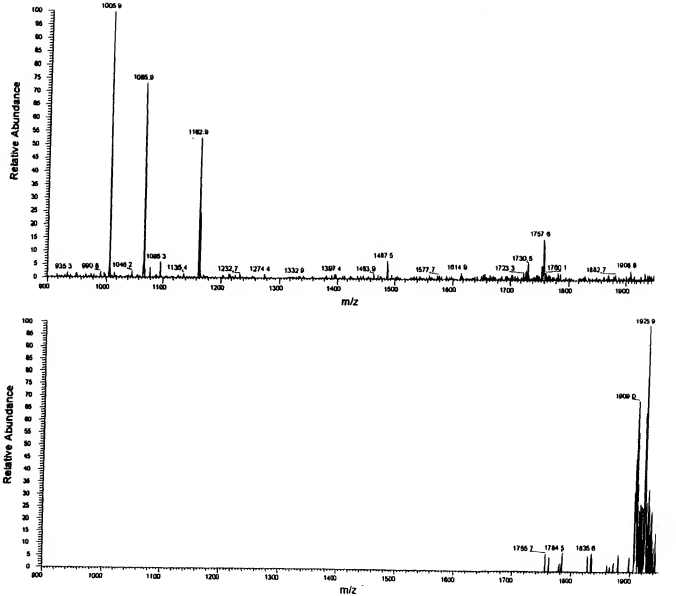


Figure 2. MS/MS of control RNA/DNA (upper); control+paromomycin (lower)

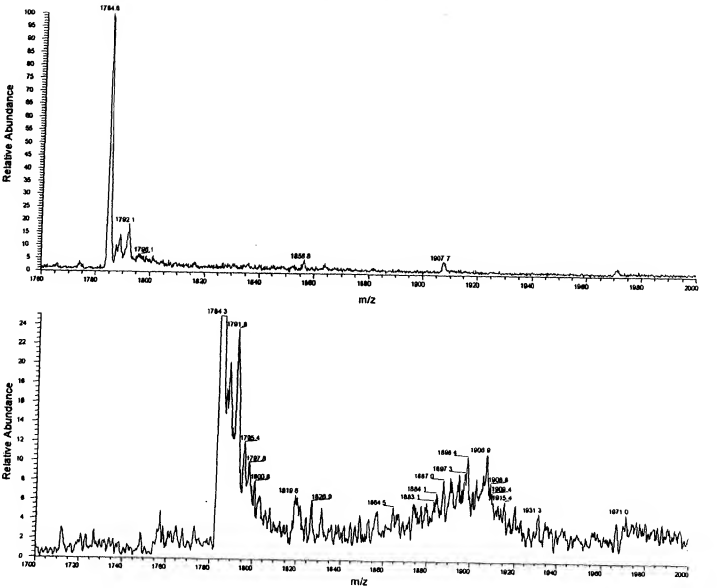


Figure 3. RNA/DNA chimera+paramomycin (upper); chimera+library (lower)

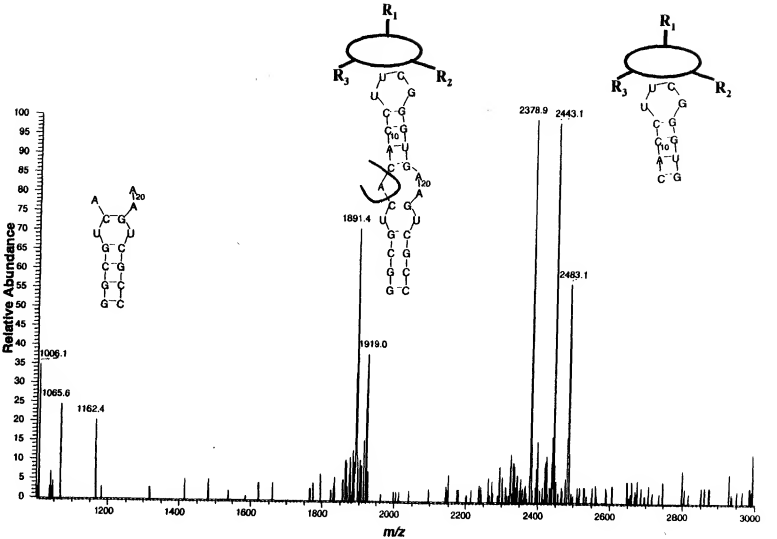


Figure 4. MS-MS analysis of member bound to RNA/DNA chimera

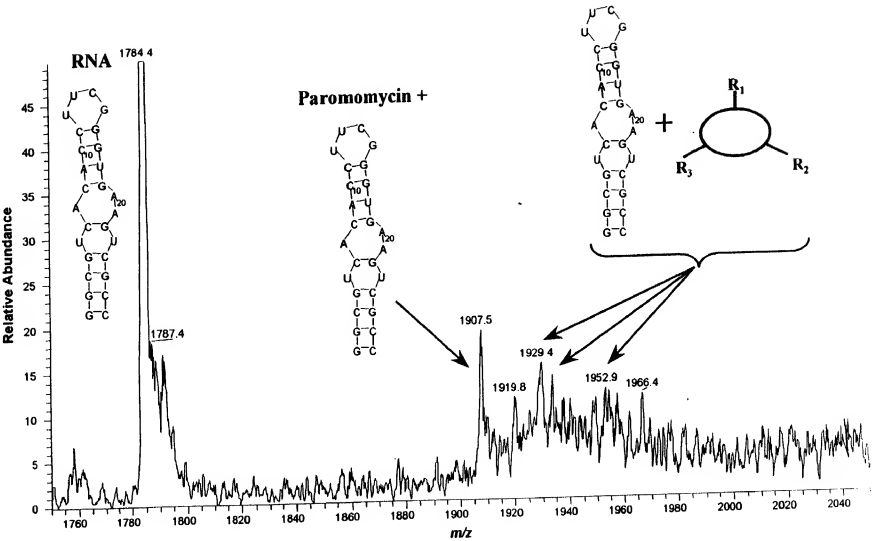


Figure 5. ESI-MS of RNA/DNA chimera bound to paromomycin and library

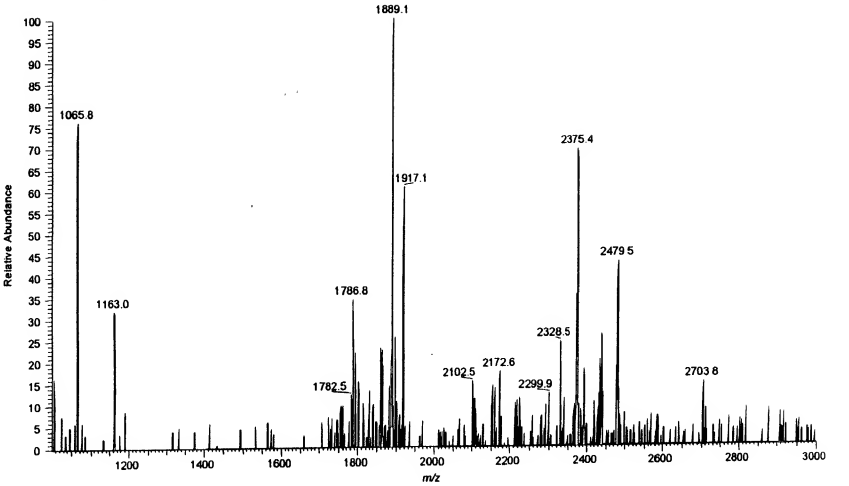


Figure 6. MS/MS of RNA/DNA chimera + compound with mass 665.1 not bound at the A-site

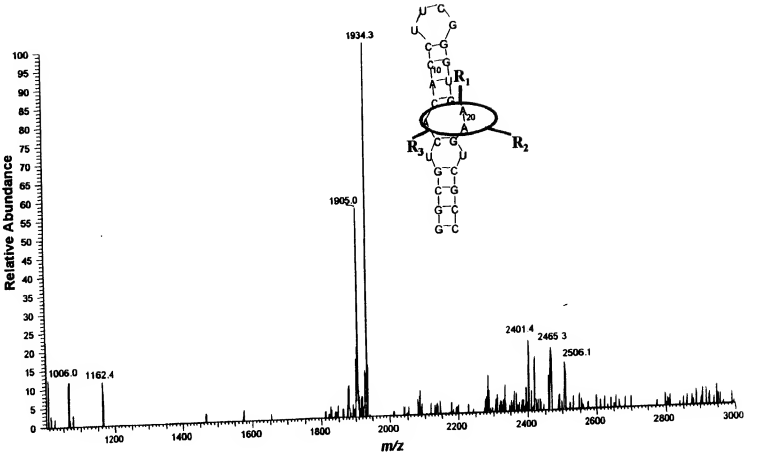


Figure 7. MS-MS analysis of member bound to RNA/DNA chimera at the A-Site

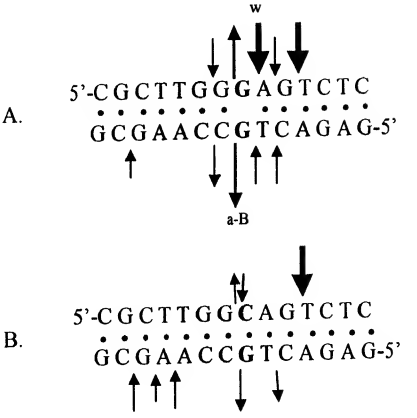


Figure 8. MS Fragmentation of DNA:DNA duplexes

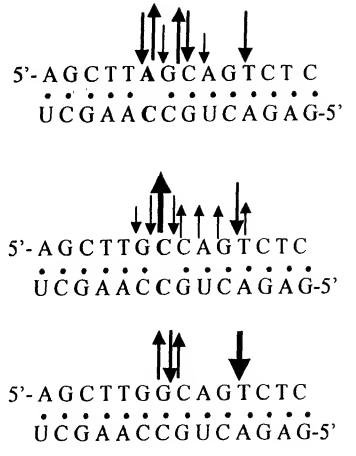
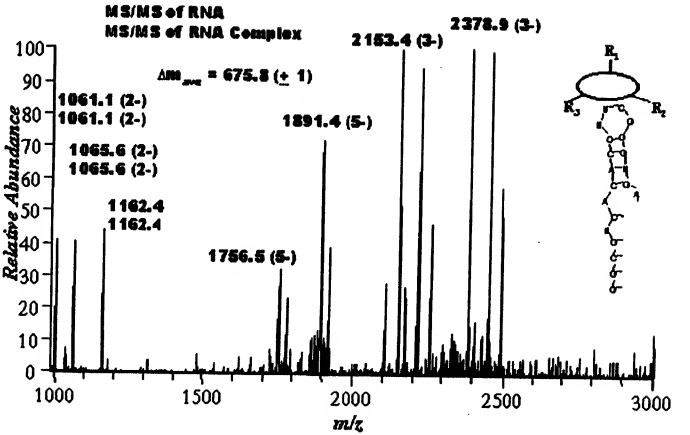


Figure 9. MS Fragmentation of DNA:RNA duplexes

MASS Analysis of Binding Location

non-A site binder

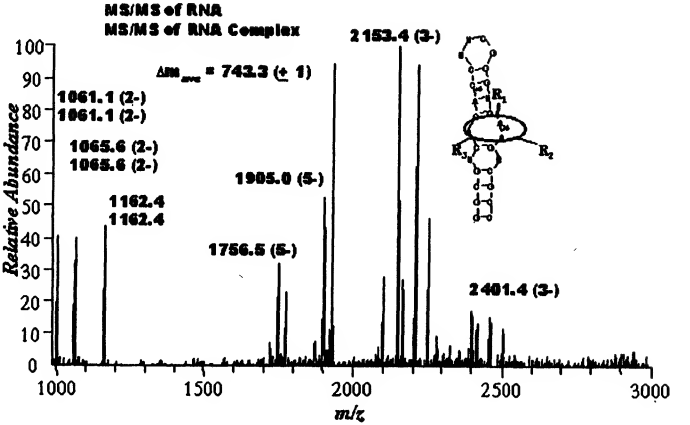
FIGURE 10



MASS Analysis of Binding Location

non-A site binder

FIGURE 11



MASS analysis of 16S A site RNA plus 216 member library

FIGURE 12

(performed on quadrupole ion trap)

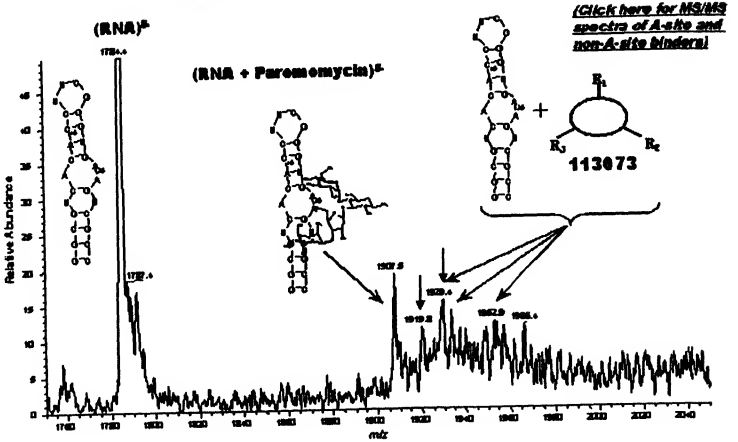


Figure 13

High Precision ESI-FTICR Mass Measurement of 16S A site RNA/Paromomycin Complex

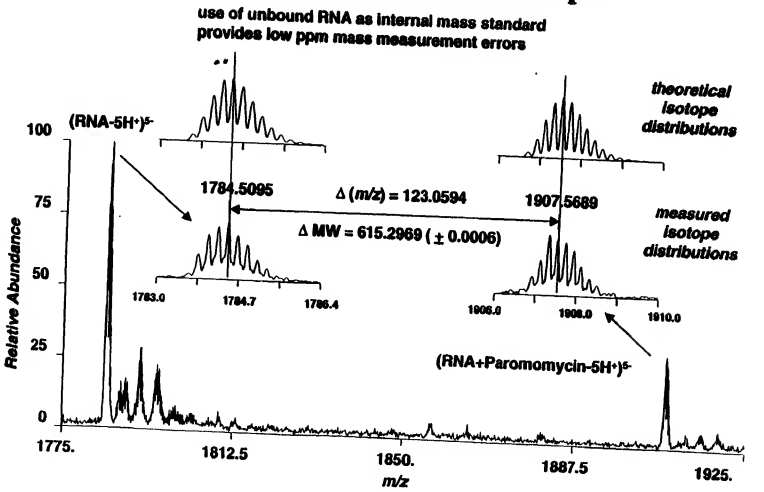


Figure 14

MASS of 60-Member Ibis Library Against 16S A-site RNA

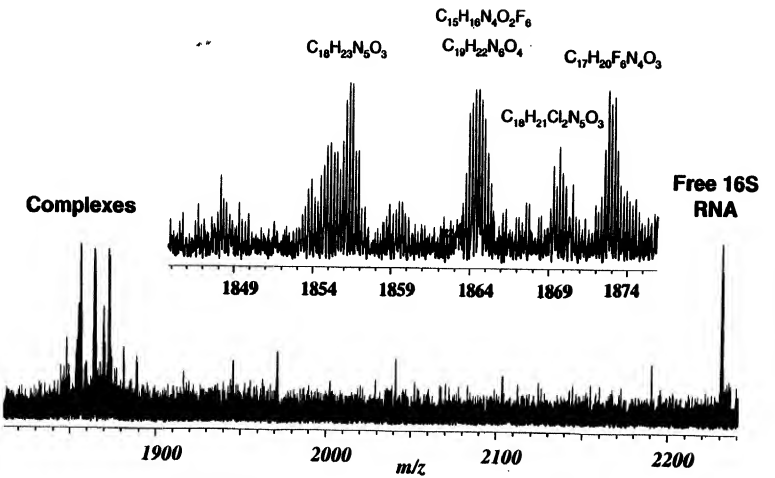


Figure 15

MASS of 60-member Library against 16S A-site Model

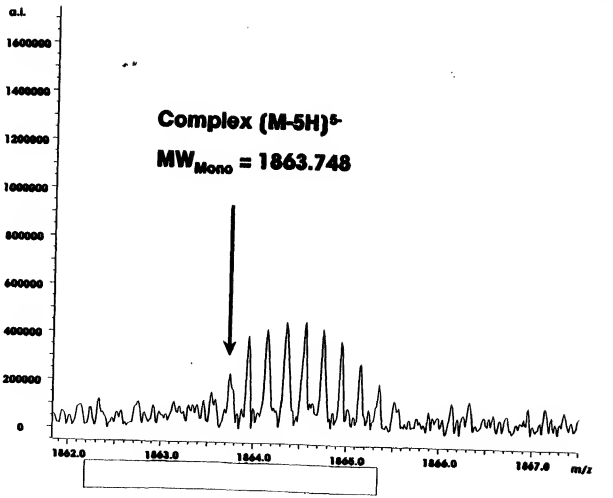


Figure 16

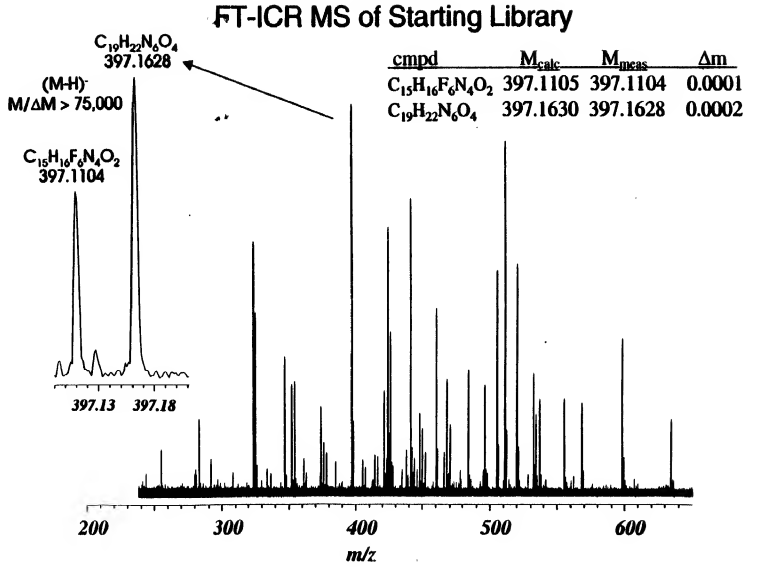


Figure 17

Compound Identification from a 60-member Combinatorial library with MASS

Complex M_{meas}	**9320.300±.009 Da**
RNA M_{meas}	**8922.189±.009**
ΔM	**398.111±.009 Da**

$C_{15}H_{16}N_4O_2F_6$	**398.117 Da**
~~$C_{19}H_{22}N_6O_4$~~	~~398.163~~

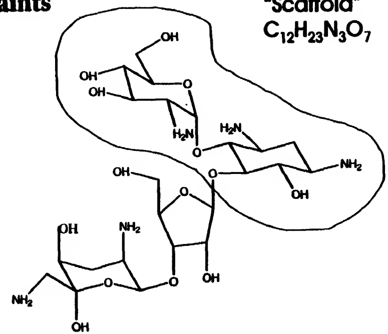
Figure 18

Elemental Composition Constraints

Measured Mass: 615.2969
Mass Tolerance: 1.0 ppm
Charge: 0

Element	Min. atoms	Max. atoms
^{12}C	12	30
^{1}H	23	60
^{16}O	7	20
^{14}N	3	20

"Scaffold"
$C_{12}H_{23}N_3O_7$



Possible Elemental Compositions:

Calc. Mass (amu)	Error (ppm)	Molecular Formula
~~615.296291~~	~~0.98~~	~~$^{16}O_4$ $^{14}N_{19}$ $^{12}C_{21}$ $^{1}H_{33}$~~
615.296298	0.98	$^{16}O_9$ $^{14}N_{12}$ $^{12}C_{22}$ $^{1}H_{39}$
615.296305	0.97	$^{16}O_{14}$ $^{14}N_5$ $^{12}C_{23}$ $^{1}H_{45}$
~~615.296808~~	~~0.15~~	~~$^{16}O_{15}$ $^{14}N_{17}$ $^{12}C_8$ $^{1}H_{41}$~~
~~615.296815~~	~~0.14~~	~~$^{16}O_{20}$ $^{14}N_{10}$ $^{12}C_9$ $^{1}H_{47}$~~

Further constrain by elemental composition of "letters"

unintended products...

Figure 19

MASS K_d determination for 16S-Paromomycin

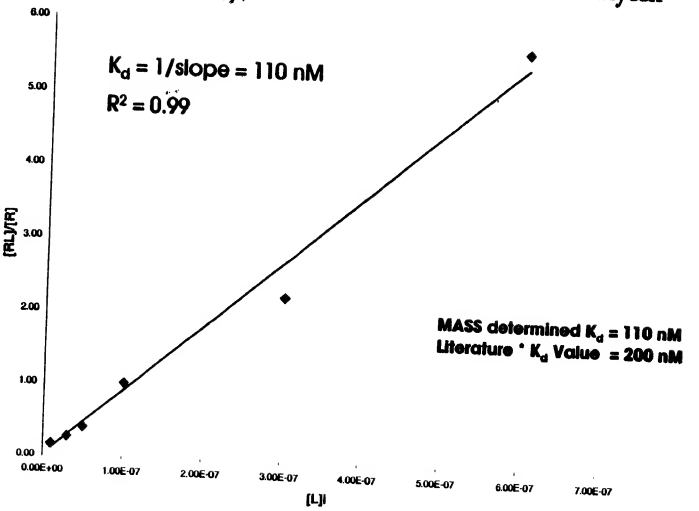


Figure 20

Multitarget Affinity/Specificity Screening

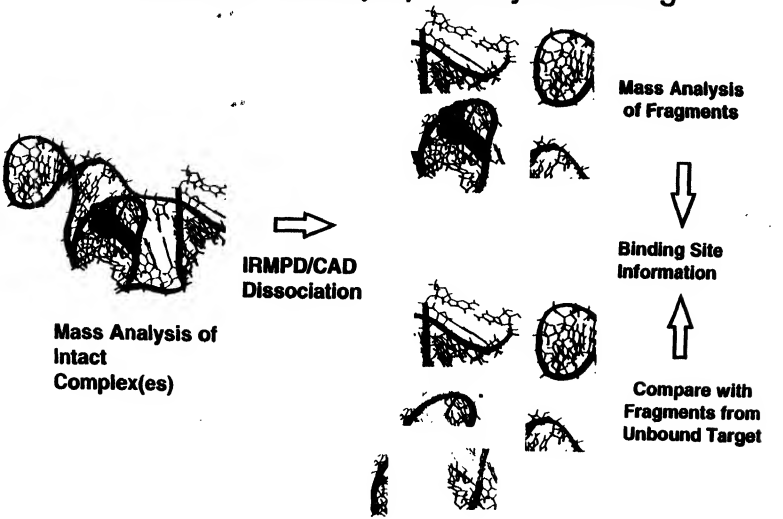


Figure 21

MASS Analysis of 27 Member Library with 16S A-site RNA

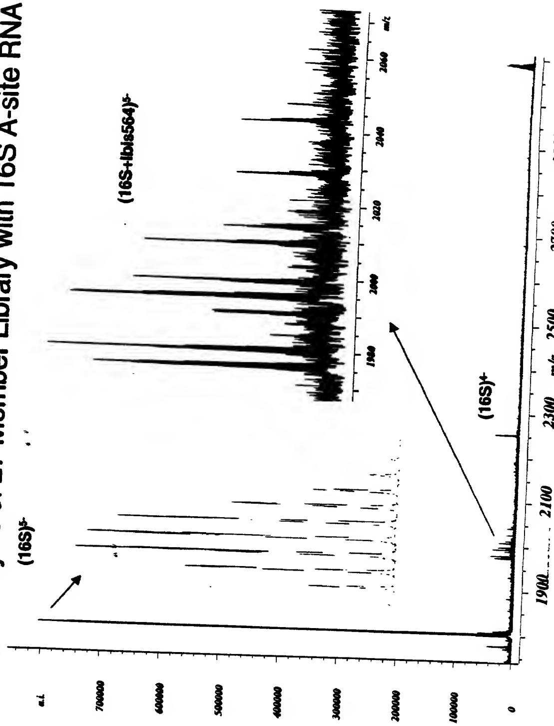
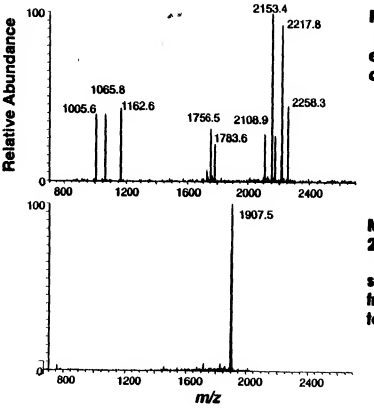


Figure 22

MASS Protection Assay



MS/MS of 16S 27-mer

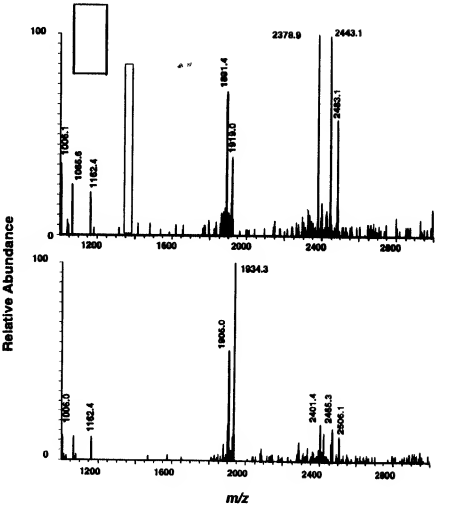
extensive fragmentation observed at weak linkages

MS/MS of 16S 27-mer + Paromomycin

significant protection from fragmentation due to paromomycin binding

Figure 23

MASS Protection Assay



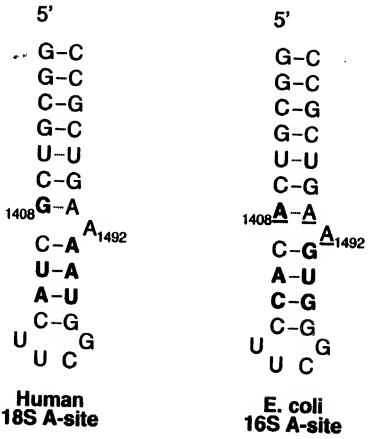
No protection
- Binding not at A-site

Reduced Fragmentation
- Binding at A-site

Figure 24

Eukaryotic and Prokaryotic A-Sites

Aminoglycoside antibiotics bind to A-site of decoding region in 16S RNA



Δ MW = 15.011 Da

Figure 25

Neutral Mass Tag Does Not Affect Ligand Binding

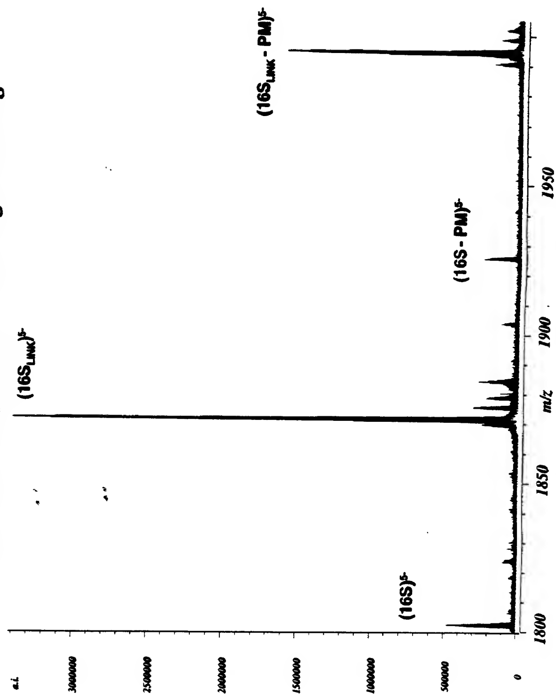


Figure 26

Simultaneous Screening of 16S A-site and 18S A-site Model RNAs Against Aminoglycoside Mixture

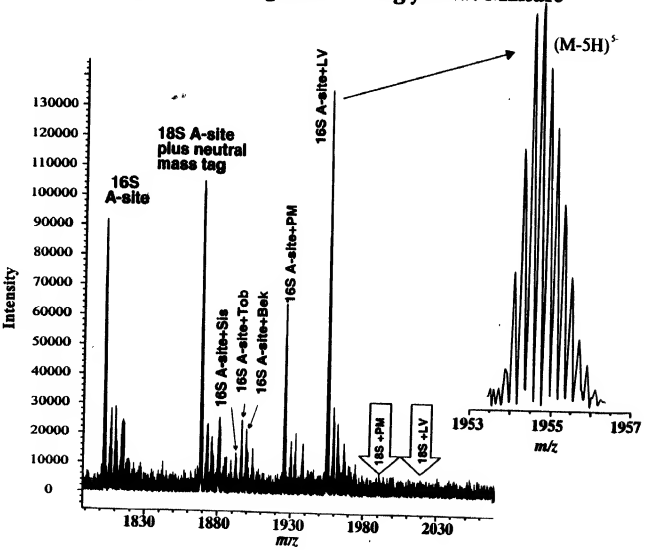
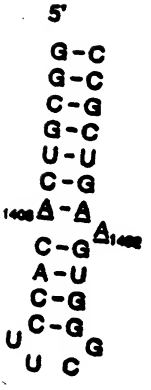


Figure 27



R A = adenosine

C A = deoxyadenosine

Figure 28

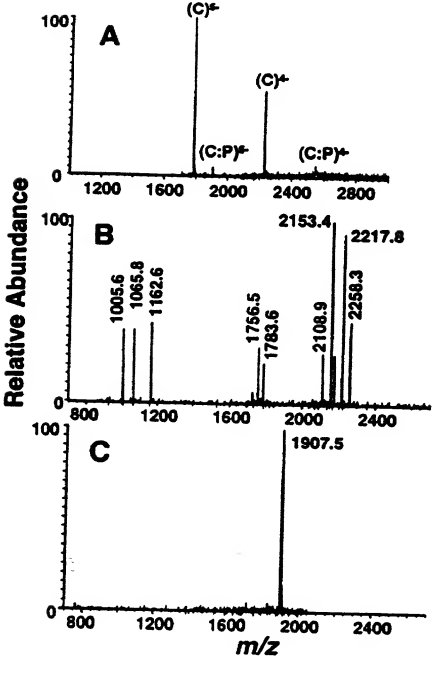
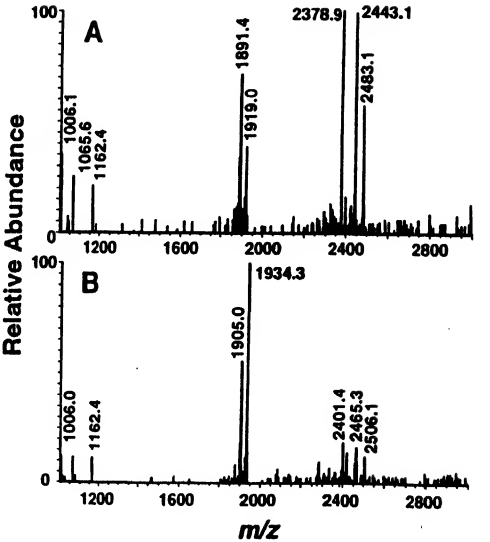

A
(C)4-
(C)3-
(C:P)4-
(C:P)3-
100
0
1200
1600
2000
2400
2800
B
2153.4
2217.8
1005.6
1065.8
1162.6
1756.5
1783.6
2108.9
2258.3
800
1200
1600
2000
2400
C
1907.5
Relative Abundance
m/z

Figure 29



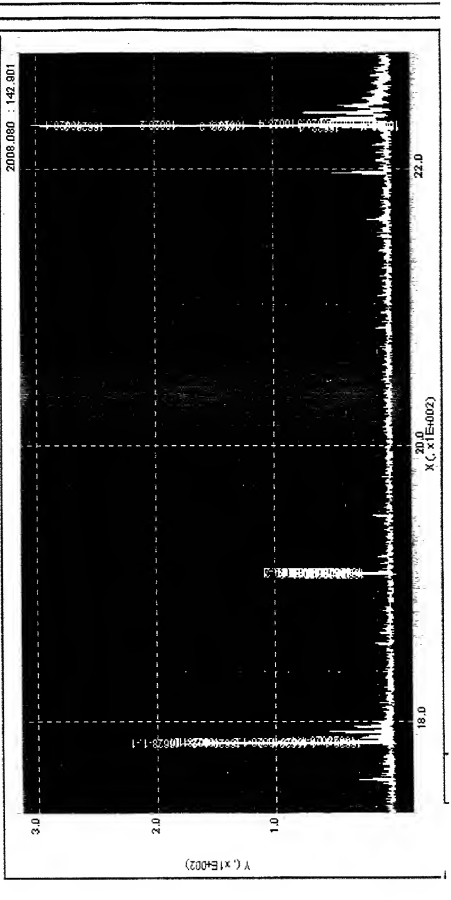


Figure 30

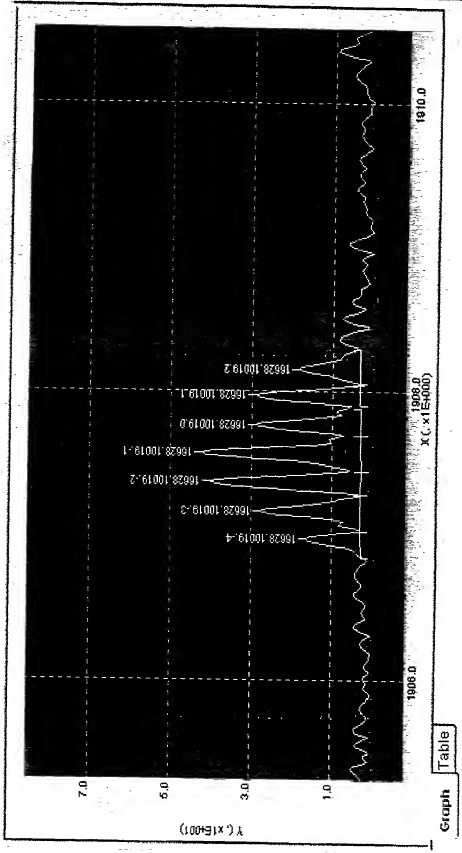

16628.10019.2
16628.10019.1
16628.10019.0
16628.10019.-1
16628.10019.-2
16628.10019.-3
16628.10019.-4
1910.0
1908.0
1906.0
X (, x1E+000)
7.0
5.0
3.0
1.0
Y (, x1E+001)
Table
Graph

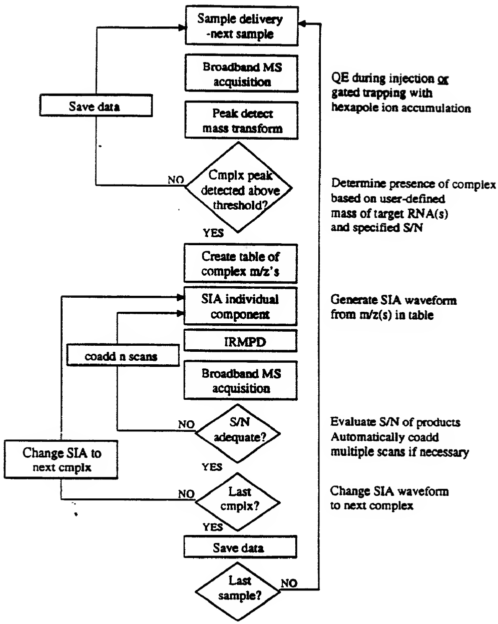
Figure 31

Figure 32

nr	name	apex	start	stop	height	area
1	16628-1.-4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.-3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.-2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.-1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628.10019.-4	1906.974	1906.874	1907.031	12.63	1.00
12	16628.10019.-3	1907.173	1907.045	1907.273	22.54	2.11
13	16628.10019.-2	1907.373	1907.287	1907.444	33.86	2.91
14	16628.10019.-1	1907.572	1907.458	1907.701	34.87	3.30
15	16628.10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628.10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628.10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628.-4	2229.874	2229.679	2230.029	27.51	4.87
19	16628.-3	2230.146	2230.029	2230.263	111.72	16.23
20	16628.-2	2230.380	2230.263	2230.516	225.18	32.39
21	16628.-1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Graph Table

Figure 33


Sample delivery -next sample
Broadband MS acquisition
Save data
Peak detect mass transform
QE during injection or gated trapping with hexapole ion accumulation
Cmplx peak detected above threshold?
NO
YES
Determine presence of complex based on user-defined mass of target RNA(s) and specified S/N
Create table of complex m/z's
SIA individual component
Generate SIA waveform from m/z(s) in table
IRMPD
coadd n scans
Broadband MS acquisition
S/N adequate?
NO
YES
Evaluate S/N of products Automatically coadd multiple scans if necessary
Change SIA to next cmplx
Last cmplx?
NO
YES
Change SIA waveform to next complex
Save data
Last sample?
NO